4 VALIDATION OF PCR- BASED ANALYTICAL PROCEDURES

The validation of new DNA technologies/methodologies utilized by the Virginia Division of Forensic Science, Forensic Biology Section is addressed in the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual, Section VI, Quality Assurance Program DNA Typing of Biological Materials. In addition, a summary of each validation study is maintained with the data. The summary and data are maintained by the Section Chief of the Forensic Biology Section as directed by Section 17 of the Quality Manual, Technical Procedures and Manuals.

4.1 Developmental Validation

During the development of a DNA procedure and prior to the adoption of the procedure by the DNA laboratory, validation studies must have been conducted by the scientific community. These validation studies form the basis for evaluating the validity, accuracy, precision and reproducibility of a particular DNA analytical procedure. The developmental validation and scientific literature should include the following:

4.1.1 Characterization of the DNA loci used for PCR Analysis

4.1.1.1 Inheritance

DNA loci used in forensic testing shall have been validated by family studies to demonstrate that the loci exhibit Mendelian inheritance as reported in scientific communications/literature. Those DNA loci used in parentage testing should have a low frequency of mutation and/or recombination (no greater than 0.2%).

4.1.1.2 Gene Mapping

The chromosomal location of the polymorphic loci used for forensic testing shall be submitted to or recorded in the Yale Gene Library or by the International Human Gene Mapping Workshop. The characterization of the individual loci is addressed in the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual, Section III, Fluorescent Detection PCR-Based STR DNA Protocol PowerPlex® 16 BIO System, Chapter 9, Interpretation of PowerPlex® 16 BIO PCR Amplification Results.

4.1.1.3 Documentation

The polymorphic loci shall be documented in the literature stating the polymerase and the primers used to detect the polymorphism.

4.1.1.4 Detection

The molecular basis for detecting the polymorphic loci shall be documented in the scientific or technical literature.

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4.1.2 Validation Studies

Validation studies addressing species specificity, sensitivity, stability and mixture studies must be conducted at minimum for each PCR-base system or individual PCR locus.

4.1.3 Population Studies

Population distribution data must be established for different racial and/or ethnic groups. The population distribution data will include the allele distribution for the locus or loci obtained from the relevant populations.

4.2 Internal Validation of Established Procedures

Prior to implementing a new DNA procedure, or an existing DNA procedure developed by another laboratory that meets the developmental criteria, the Virginia Division of Forensic Science, Forensic Biology Section will validate the new PCR-based procedure internally to demonstrate the reliability of the procedure. This same prerequisite applies to any existing procedure to which significant modifications have been made. This validation forms the basis for assessing the specificity, reproducibility, and limitations of the particular PCR-based procedure. The validation shall include the following:

4.2.1 The PCR-based method will be tested using known samples and non-probative evidence samples.

4.2.2 If developmental validation studies have not been conducted or are not available in the scientific literature prior to the implementation of the PCR-based procedure for analyzing casework or Data Bank samples, the following studies will be conducted at minimum:

4.2.2.1 Standard Specimens (Tissue and Aged Studies)

The typing procedure will be evaluated using fresh body tissues and fluids that have been obtained and stored in a controlled manner for varying lengths of time. The study will be used to determine that DNA isolated from different tissues from the same individual yields the same typing profile and the stability of DNA for aged samples.

4.2.2.2 Consistency/Sensitivity (Dilution Study)

Using specimens obtained from donors of known type, the reproducibility of the technique will be determined using different concentrations of DNA.

4.2.2.3 Population Studies

Population distribution data will be established for different racial and/or ethnic groups. The population distribution data will include the allele distribution for the locus or loci obtained from the relevant populations. The population frequencies for each of the relevant populations will be maintained as part of the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual, Section III, Fluorescent Detection PCR-Based STR DNA Protocol PowerPlex® 16 BIO System.

4.2.2.4 Mixed Specimen Studies (Mixture Study)

The ability of the system to detect the components of mixed specimens and define the limitations of the system will be investigated.

4.2.2.5 Time/Temperature Studies (Environmental Study)

A determination will be made as to whether the polymorphic patterns in dried stains change as a function of time and temperature.

4.2.2.6 Degradation Studies (Substrate and Contamination Studies)

Laboratory prepared body fluid stains will be exposed to a variety of commonly encountered substances and contaminants to assess the impact of these factors on DNA profiles.

4.2.2.7 Non-Probative Evidence (Non-Probative Case Study)

DNA profiles in non-probative evidentiary stain materials will be examined. The DNA profiles obtained for a known blood sample versus a questioned body fluid deposited on typical crime scene evidence will be compared.

4.2.2.8 Nonhuman Studies (Animal Study)

A determination will be made as to whether DNA typing methods designated for use with human specimens detect DNA profiles in nonhuman source stains.

4.2.2.9 Inheritance Studies (Family Study)

DNA loci used in forensic testing shall have been validated by family studies to demonstrate that the loci exhibit Mendelian inheritance.

4.2.2.10 Precision Studies (Precision of PCR-Based Instrument)

The Forensic Biology Section will conduct precision studies on each instrument used to conduct the PCR-based analysis to establish a sizing window based upon empirical data.

4.2.3 Before the PCR-based procedure is used by a casework examiner he/she will successfully complete a qualifying test.

4.2.4 If a significant modification has been made to an analytical procedure, the modified procedure must be compared to the original using identical types of samples.

♦**END**